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MODE OF OPERATION OF A SYSTEM OF CONTROLLING ELEMENTS IN MAIZE

1. Introduction
2. Origin of a_1^{m-1}
3. Outline of mode of operation of the Spm-a_1^{m-1} system
4. States of a_1^{m-1}
5. Detection of the Spm element and its mode of operation
6. Responses of different states of a_1^{m-1} to the same Spm element
7. Transposition of Spm
8. Stability of Mutants
9. Types of Spm elements
10. Modifier element in the Spm system
11. Conclusions

Mode of Operation of a System of Controlling Elements in Maize.

General descriptions of controlling elements in maize and of their modes of operation have been given in a number of reports appearing in recent years (for literature citations, see McClintock, 1956b). It is the purpose of t is report to consider some of the experimental methods that have been employed to discover the mode of operation of the system of controlling elements which has been referred to in other publications as the Spm (Suppressor-mutator)-a_1^{m-1} system. Controlling elements may be defined as those unit components, carried in the chromosomes, that serve to control gene action both with regard to type and degree and to the tissues or parts of a tissue ~~where~~ in which this will occur. The different controlling elements are recognized by means of their distinctive modes of control of gene action, regardless of the primary type of action of the gene substance itself. They exhibit Mendelian inheritance patterns and their locations in the chromosome complement may be determined by use of ordinary genetic techniques. However, they may undergo change in location within the chromosome complement, appearing at new locations and disappearin from previously determined locations, without losing their distinctive properties in the process. This process has been termed transposition and

the several methods that have been used to detect such transposition were presented previously (McClintock, 1956b).

For many years, efforts of the author were concentrated on analysis of the system composed of the two controlling elements designated Ac (Activator) and Ds (Dissociation). This system was chosen for extended studies because both elements ~~of this system~~ in it could be identified readily and regardless of the location in the chromosome complement that each might occupy. Thus, change in location of these elements, and the effects produced when one of them is inserted at the locus of a known gene, could be detected and subsequently analysed. Altogether, the operation of this system at eight ~~known~~ gene loci has been examined. Also, two or more independent insertions of one of these elements at four of these eight gene loci were detected and the consequence of this examined in each case. It was concluded from these studies that this system should be able to operate at any gene locus provided that the effects of ~~its operation~~ this at a particular ~~gene~~ locus is not lethal. Most important, however, is the realization that the mode of control of gene action may be predicted in advance, for it will follow the discovered rules that ~~characterize~~ apply to the operation of this system. Knowledge of the mode of operation of the Ac-Ds system has been useful in guiding experiments aimed at revealing the mode of operation of other control system This applies particularly to methods for identifying controlling elements

and for detecting their transpositions. It has also provided a model for recognizing and subsequently appraising the changes in state of the affected gene locus. Such changes must be recognized if confusion in designing experiments and interpreting results is to be avoided.

Some years ago, a large number of different variegates appeared in the progeny of individual plants with a history of having been subjected to the breakage-fusion-bridge cycle in their early development (McClintock, 1951). At the time these variegates appeared, it was realized that it would not be possible to examine each of them. Therefore, only a few among the many that appeared were selected for subsequent study. The Ac-Ds system was discovered among one of these selected cases; and as the mode of operation of this system became apparent, attention was focused mainly upon it. Study of the other selected cases either was discontinued or it was sharply curtailed until adequate time could be found for more detailed examination. The system responsible for control of gene action in one of these latter cases is now sufficiently understood to allow conclusions to be drawn regarding the types of controlling elements that are involved in it and their modes of operation. This is the system ~~responsible for control of gene action~~ operating at both a_1^{m-1} and a_2^{m-1}, mentioned in previous reports. In this report, attention will be given mainly to a_1^{m-1}.

Origin of a_1^{m-1}

The history of origin of a_1^{m-1} from a modification that occured at a standard A_1 locus is as important for an appreciation of the controlling elements involved in this system and their modes of operation as were the histories of origin of modified gene action that appeared in the Ac-Ds carrying cultures for an appreciation of the presence and mode of operation of the elements of that system. The modified A_1 locus, designated a_1^{m-1}, was the third recognized case of change in gene action in a sequence which commenced with an alteration at a previously unidentified gene locus concern with chlorophyll production. The plants having this first member of the sequence exhibited variegation for chlorophyll pigmentation. This variegat was one of those originally selected among the many that appeared in the original cultures, as described above. In the early stages of examination of this variegate, a number of plants in one culture were self-pollinated. On the ear produced by one of these plants, some kernels appeared that exhibited variegation for anthocyanin pigmentation. Spots of deep pigmentation appeared in a colorless background. The plants derived from them also exhibited variegation for anthocyanin pigmentation. Subsequent tests of these plants and their progeny indicated that an alteration had occurred at the standard A_2 locus in one chromosome 5 of the parent plant. This altered locus was given the designation a_2^{m-1}. Tests were then undertaken to examine the changes in expression of gene action at a_2^{m-1} and t

determine the factors involved in control of this. In the course of this study, a number of plants in a culture in which the system responsible for control of gene action at a_1^{m-1} was present, were used as pistillate parents in crosses with plants that were homozygous for the standard recessive, a_1, in chromosome 3, and for the standard A_2 locus in chromosome 5. On one of the ears this cross produced, a single kernel was found that exhibited spots of anthocyanin pigment in a non-pigmented background. A plant was grown from this kernel and this plant, in turn, exhibited variegation for anthocyanin pigmentation. ~~As expected,~~ Tests crosses utilizing this plant both pollen and ~~ears of this plant~~ indicated the presence in it of a modified ~~altered~~ A_1 locus, and ~~it~~ the altered locus was thereupon designated a_1^{m-1}. It was evident that some change ~~the alteration~~ had occurred at ~~had occurred at~~ the standard A_1 locus in one chromosome 3 in the pistillate ~~female~~ parent plant, and this took place late in development of one of the ears ~~of~~ this plant, for only one kernel on this ear exhibited altered A_1 action. Studies aimed at determining the components of the system responsible for control of gene action at both a_1^{m-1} and a_2^{m-1} were contined but initially only on a limited scale. Only recently has time been found to examine this more completely. It is now known that the same system of control of gene action operates at both a_1^{m-1} and a_2^{m-1}. Very likely this same system also operated to control action of the gene that was associated with chlorophyll production,--the initial variegate in the sequence. This postulate is based ~~only~~ solely upon the patterns of variegation that were exhibited and upon inheritance behavior, for study of this case was discontinued some years ago

and therefore direct tests to determine identity of control systems cannot now be made. Outline of the modes of operation of the Spm-a,$^{m-1}$ Spt

In order to learn of the system that is responsible for control of gene action at a_1^{m-1} and a_2^{m-1}, a large number of different types of tests were conducted. The results obtained from each are consistent with one another on the basis of the eventually determined modes of operation of the componen of this system. In order to comply with space requirements, only a selecte set of tests ~~will~~ can be ~~given~~ mentioned here. These are chosen in order to illustrate t salient features of the mode of operation of this system and they will be confined to studies conducted with a_1^{m-1}. Those conducted with a_2^{m-1} will be given in a separate report.

From examination of the Ac-Ds system, it was learned that insertion of the Ds element at the locus of a gene initiated the primary modification that brought this action of the gene under the control of the Ds-Ac system. In many cases, the action of the gene was noticably altered by this initial event and detection of the ~~insertion~~ presence of Ds at the gene locus could be made shortly after it ~~occurred~~ was inserted there. Subsequent ~~change at~~ modifications of the locus results from the effects Ac exerts on the Ds element. The consequence of this is either removal of Ds from the locus of the gene or a modification at the locus, induced by Ds, that effects a change in its organization,--a change in state of the locus.

Both types of events can give rise to recognizable changes in action of the gene substance. With regard to a_1^{m-1}, insertion of a particular controlling element at the standard A_1 locus is considered to have occurred and to have been responsible for the initial change in gene action. Like Ds, it is this element that directly controls the type of gene action that will occur at the modified A_1 locus and also the types of change in this ~~action~~ that may occur subsequently. It appears to be the same element that is present at a_2^{m-1}. This conclusion is based on the response both ~~of~~ a_1^{m-1} and a_2^{m-1} to the presence of an independently located element designated Suppressor-mutator (Spm). When Spm is present, no action of gene is produced in either kernel or plant except in certain cells. ~~All action of the gene substance at both a_1^{m-1} and a_2^{m-1} is suppressed when Spm is present in the nuclei of a plant except in certain cells~~, in these cells where ~~a change occurs to~~ the element residing at either a_1^{m-1} or a_2^{m-1} initiates modifications that allows the gene substance to become active and this activity may be observed ~~either in this cell or~~ in the descendant cells. The particular type of expression of the gene that these modifications effect is thereafter maintained either in the presence or the absence of the Spm element. It may be concluded, then, that ~~In other words~~, the Spm element is complementary to the element located at a_1^{m-1} and a_2^{m-1}. It serves to activate it and as a consequence of this, stable mutations are produced. In this respect, it resembles Ac in the Ds-Ac system. However, the genic substance at both a_1^{m-1} and a_2^{m-1} may be active to some extent when Spm

is not present in the nucleus. When it is removed, either through meiotic segregation or by means of somatic transposition, anthocyanin pigment may appear in the kernels and plants having either a_1^{m-1} or a_2^{m-1} and its distribution is uniform. In other words, there is no variegation. The type and intensity of pigmentation is an expression of the particular state of ~~either~~ the a_1^{m-1} or a_2^{m-1} locus that is present. These states and their origins will be considered shortly. When Spm is returned to the nuclei by appropriate crosses, ~~all~~ gene action at a_1^{m-1} and a_2^{m-1} is suppressed except in those cells where mutation-producing events occur. Thus, Spm serves not only to activate the complementary element residing at a_1^{m-1} and a_2^{m-1} and thereby conditions stable mutations at those two loci, but it also must act upon this element in yet another manner for its presence results in suppression of known potentials for gene action, except in those cells where mutation-producing events occur. These two ~~seemingly~~ different aspects of the mode of control of the Spm element on the element residing at a_1^{m-1} and a_2^{m-1} are responsible for its ~~being~~ designation, Suppressor-mutator. However, this seemingly dual action may be the expression of only one process rather than of two unrelated processes, as will be indicated later.

The states of a_1^{m-1}

All examinations of the effect of a controlling element at a gene locus

must be conducted with the affected locus. Since, in addition to stable mutations, the controlling element may also initiate structural or organizational modifications of the locus that alter its subsequent expression,--that is, change the state of the locus,--it is necessary to consider states of the affected locus and their origins before detailed results of experiments ~~can be~~ are presented. With a_1^{m-1}, a changed state is readily recognized by the appearance of an individual kernel or plant that exhibits an altered response of the locus to the presence and absence of Spm. ~~For example,~~ When the controlling element first entered the locus of A_1, it effected a particular type of modification in the structure or organization of the locus. In the absence of Spm, some gene action occurred at this locus. Kernels were lightly but uniformly pigmented as were the plants derived from them. In the presence of Spm, however, all gene action was suppressed except in some cells where mutation-producing events occurred at thelocus of a_1^{m-1} that allowed the gene substance to be active in the descendants of this cell. ~~Each~~ all such event did not ~~result in~~ effect the same degree or type of gene action but many of them restored the full or near full A_1 type activity. Many of these events occurred relatively early in plant and kernel development. ~~Unlike Ac,~~ Differences ~~variations~~ in dose of Spm had no effect on altering the time of occurrence, ~~of these~~ they and in this respect the action of Spm differs from that of Ac.

events. A number of mutation-producing events occurred in germinal cells and this made it possible to examine the nature of the mutation in the next generation. Another type of change at a_1^{m-1} also occurred in a few of the germinal cells. These resulted in altered expressions of the locus both in the presence and absence of Spm. Some of them were detected initially in individual kernels in the progeny of the original a_1^{m-1} carrying plant. When this plant was crossed to plants that were homozygous for the standard recessive, a_1, which does not respond to Spm but responds, instead to Dt, the a_1^{m-1} locus in the majority of kernels that received it was unmodified. Among the kernels receiving the unmodified a_1^{m-1} locus and also Spm, the variegation pattern was much the same. There were many large pigmented areas, indicating early occurring mutation-producing events. Many of these exhibited the full A_1 type of pigmentation. However, an occasional kernel appeared that exhibited a quite different pattern of pigmented areas. Two kernels were found among several thousand that had only small pigmented areas. These were uniformly distributed and the intensity of pigment in them was either quite light or very dark. Another kernel appeared that had a number of large pigmented areas as well as some small areas but the intensity of the

pigment in all of them was ~~much less than that given by the standard A~~ relatively light. $_1$. It ranged from very faint in some areas to ~~a medium intensity~~ dark pale in others.

Plants were grown from the three described exceptional kernels. Tests conducted with them and their progeny indicated that the pattern of variegation exhibited in the presence of Spm was heritable. The altered a_1^{m-1} locus,--the changed state of the locus,--in each case responded in its own particular way to the presence of Spm and also to its absence. In the absence of Spm, the state of a_1^{m-1} ~~present~~ in the first described kernel produces ~~deeply pigmented~~ deeply pigmented kernels and plants. That in the second described kernel gives rise to very lightly pigmented kernels but rather darkly pigmented plants. The state of a_1^{m-1} present in the third described kernel produces no pigment in either plant or kernel in the absence of Spm. Subsequently, other states of a_1^{m-1} have been were isolated. Each is distinguishable from the other by the types of mutation and the time and frequency of their occurrence ~~in the presence of Spm~~ that appear when Spm is present as well as the type of expression of the gene substance that ~~occurs~~ appears in the absence of Spm. In all cases, gene action is suppressed in the presence of Spm except in those cells (and their descendants) in which mutations - Kernel sports occur. No relationship was noted among the different states between the control of time and frequency of occurence of mutation in the presence of Spm and that of type of gene action that occurs in its absence. Figure 1 illustrates the distinctiveness of several of the states of a_1^{m-1}, and Table 1 records the range of differences that is expressed among the presently isolated states of a_1^{m-1}.

The integrity of a state of a_1^{m-1} is maintained in heterozygous plants and this applies to plants that carry a different state in each of their chromosomes 3. In such plants, each state responds to Spm in its own predictable way and the variegation patterns each produces will be expressed in the plant or kernel tissues. Figure 2 illustrates this. The kernels in the photographs carry the state shown in — of figure 1 in one chromosome 3 and the state shown in — of figure 1 in the homologue. The pattern of mutation and the mutation type produced by each state are readily recognized in these kernels. In the plants, normal segregation of the two states occurs at meiosis and each may be recovered in the expected proportion from the gametes that these plants produce.

States of a_1^{m-1} are maintained unchanged in the absence of Spm. In its presence, however, new states may arise and the frequency of occurrence of this in germinal cells is related to the time of occurrence of mutation-producing events that a particular state exhibits. If the state produces some early occurring mutations, then new states of a_1^{m-1} may appear in the germ cells, and the frequency of this is proportional to the frequency of occurrence of these early mutations. If, on the other hand, the state is one in which mutations occur only late in development of the plant, ~~or kernel,~~ then few or no altered states may be recovered in the gametes of

these plants. The mutation-producing events at a_1^{m-1}, regardless of state, give rise to stability of expression of the locus. The particular type of gene action the mutational event produces continues to be expressed in subsequent generations both in the presence and in the absence of Spm. This suggests that the change responsible for these mutations may ~~have~~ removed the controlling element from the locus or it may ~~have~~ resulted in its inactivation with regard to Spm. Since the changes in state arise at the very same developmental period as the mutation-inducing events occur, it is concluded that they represent a modification at the locus induced by the controlling element residing there that did not result either in its removal or inactivation. In other words, the element responded to the presence of Spm at the appointed time and in the appointed cells but the consequence of this was not the usual one,--i.e., the mutation-producing event. Instead, the responding element itself was either modified or it induced some reorganization at the locus that modified its capacity to respond to Spm with regard to time + frequency of occurrence of mutations as well as ~~when this will occur~~, the types of mutations it can induce, and also with regard to ~~as well as~~ the type of gene action that can occur in the absence of Spm. It is as evident in this case as it is with Ds that a change in state is one of the consequences of the response of the

of the controlling element residing at a gene locus to the independently located element of the system,--Spm in this case and Ac in the case of Ds.

Detection of the Spm element and its mode of operation

~~Transpositions of Spm~~

In the early examination of a_1^{m-1}, no evidence was found of the presence of the Spm element, ~~was detected~~. This was because the original a_1^{m-1} carrying plant had a number of Spm elements located at various positions in the chromosome complement. All or certainly nearly all of the gametes it produced had Spm elements ~~in~~ them. Since the dose of Spm has no appreciable effect on the pattern of mutation produced by a_1^{m-1}, differences in number of Spm elements in a kernel or plant is not made directly evident by this means as it is with Ac. It was only after several generations of crossing of a_1^{m-1} carrying plants to a_1 tester stocks which did not have Spm, that ~~definite~~ clearly expressed Mendelian type ratios of variegated to uniformly pale ~~light~~ colored kernels appeared on the test cross ears. These ratios indicated the presence in the a_1^{m-1} carrying plants of an independently located element that is associated with control of a_1^{m-1} expression. They also indicated that the number and the location of this element was not the same in all tested plants. In the meantime, several different states of a_1^{m-1} had been isolated on the basis of the altered variegation patterns that appeared in individual kernels on several of the ~~first~~ testcross ears, as described earlier. In successive generations of crossing of

plants carrying these different states of a_1^{m-1} to the a_1 tester stocks, the same kinds of ratios of variegated to non-variegated kernels also began to appear. It was then assumed that the uniformly pigmented (non-variegated) kernels and plants carried a_1^{m-1} but not the independently located element. On the other hand, this element was assumed to be present in those kernels and plants that showed pigmented areas in a non-pigmented background. On this interpretation, the independently located element was exerting a suppressor-mutator type of control of gene action at a_1^{m-1}. Since the dose of this element obviously did not affect the pattern of mutational events, control of this must reside at the a_1^{m-1} locus itself, the type depending upon the state of the locus. It was evident that the phenotypes of the nonvariegated kernels and plants also reflected the state of the a_1^{m-1} locus that was present in them.

The hypothesis stated above was subject to test. If it were correct, evidence in support of the following four statements should be obtained:

(1) All variegated kernels and plants carry at least one Spm element.

(2) No Spm element is present in the non-variegated class of kernels and plants. (Germinal mutations, ~~described~~ mentioned earlier, are excluded from this class.)

(3) The a_1^{m-1} locus in the non-variegated kernels and plants is capable

of responding to Spm if this element is subsequently introduced into a nucleus.

(4) The type of response to Spm and the phenotypes produced in its absence is a function of the state of the a_1^{m-1} locus.

A large body of evidence in support of these statements is now available and it has been obtained by various types of test, only a few of which need be outlined here.

In order to facilitate identification of the presence or absence of Spm in a particular plant, so-called Spm tester stocks were developed. These stock have either one or the other of the two states of a_1^{m-1} shown in (sr1) and (sr1a8-1) of figure 1, and these states were selected for the following reasons. In the first place, when Spm is present, very few germinal mutations or changes in state occur. Therefore, nearly all of the gametes produced by plants carrying these states of a_1^{m-1} and also Spm have an unmodified a_1^{m-1} locus in them. Secondly, the pattern of variegation each produces in the presence of Spm is distinctive and non-obscuring. Thirdly, in the absence of Spm, one of these states, (sr1a8-1) figure 1, gives rise to darkly pigmented kernels and this is a useful character in some tests. The tester stocks were made homozygous for one or the other of these two states. They were also made homozygous for either Sh_2 or sh_2, located

very close of a_1^{m-1} (about one-quarter of a percent crossing over occurs between them).

An example of one series of tests will illustrate some of the methods employed to determine Spm constitutions of individual plants. The silks of two ears of a variegated plant carrying a_1^{m-1} and Sh_2 in one chromosome 3 and a_1 and sh_2 in the homologue and also Y in one chromosome 6 and y in its homologue, recieved pollen from a plant that was homozygous for a_1, sh_2 and y. The state of a_1^{m-1} in the pistillate plant was that shown in [illegible] of figure 1. From this cross, the two ears produced a total of 745 kernels. There were 181 Sh_2 kernels in which the aleurone layer was uniformly and rather darkly pigmented; 69 of these were Y and 112 were y. The aleurone layer in another 188 Sh_2 kernels exhibited a number of spots of the full A_1 type pigment in a colorless background and 117 of these were Y and 71 were y. The aleurone layer in the remaining Sh_2 kernels was completely colorless and the starch in its endosperm was y. Among the 375 sh_2 kernels on these two ears, the aleurone layer in 373 of them was totally colorless; 186 of these kernels were Y and 187 were y. The remaining 2 sh_2 kernels exhibited spots of full A_1 type pigment in a colorless background. The phenotype of the starch in one of them was Y and that in the other was y. Since

a_1^{m-1} is colsely linked to Sh_2, nearly all of the Sh_2 class of kernels on these two ears should carry an a_1^{m-1} locus and nearly all of the sh_2 kernels should be homozygous for the standard recessive, a_1. The close linkage of a_1^{m-1} to Sh_2 is obvious for only 1 Sh_2 kernel in the total of 370 was completely colorless and only 2 sh_2 kernels in a total of 375 had pigment in the aleurone layer.

On the basis of the interpretation given above, the uniformly pigmented kernels should have no Spm in them whereas those exhibiting spots of the full A_2 type pigment in a colorless background should have this element. From the ratio of these two classes among the Sh_2 kernels (181 to 187) it could be concluded that the variegated pistillate parent plant had one Spm. The ratio of Y to y in each of these two classes indicated that this Spm element was carried in the Y bearing chromosome. It was then necessary to determine whether or not these conclusions were valid. For this purpose, 104 plants were grown from various types of kernels on these two ears and tests were conducted with them. The phenotypes of the selected kernels were as follows: 11 uniformly pigmented Sh_2 Y, 13 uniformly pigmented Sh_2 y, 17 variegated Sh_2 Y, 8 variegated Sh_2 y, 1 variegated sh_2 Y, 30 colorless sh_2 Y, and 24 colorless sh_2 y. All 24 plants derived from the uniformly pigmented kernels were themsleves

uniformly pigmented. All ~~of the~~ 26 plants derived from the variegated kernels showed small streaks of the A_1 type pigment in a non-pigmented background. And, as expected, all 54 plants derived from the colorless sh_2 class of kernels lacked anthocyanin pigment. Each plant was then tested for presence or absence of Spm by crossing it with a plant in an Spm tester stock.

To illustrate how the tester stocks can serve to reveal the presence or absence of Spm, those tests conducted with the 54 plants derived from the colorless, sh_2 class of kernels will be considered first. This is a completely objective test since the presence or absence in any one of them of Spm could not be assumed on the basis of phenotypic expressions. The silks of one or more ears of each of these plants received pollen from plants that were homozygous for either state - or - of figure 1, for Sh_2 and for y; these pollen parents were uniformly pigmented indicating the absence of Spm in them according to the stated hypothesis. If the plant being tested has no Spm, then all of the kernels on the resulting ear will be uniformly colored. If, however, the plant being tested carries Spm, then it should be present in some of its gametes. Following introduction of the a_1^{m-1} locus from the male parent, the presence of Spm in those kernels that received it from the female parent should be revealed by the appearance in them of small,

deeply pigmented spots in a colorless background due to activation of the a_1^{m-1} locus by the Spm element. In those kernels that did not receive Spm, the aleurone layer should be uniformly pigmented. Among the 30 plants derived from the colorless, sh_2, Y class of kernels, it could be determined on this basis that 15 had a single Spm element and 15 had no Spm. In 13 of the 15 plants that had Spm, linkage of it with Y was evident (A, table 2) but in the 2 remaining plants, no linkage of Spm with Y was evident (B, table 2). (The reason for the absence of linkage of Spm with Y in these 2 plants will be considered later. It need only be mentioned here that this is not unexpected.) Among the 24 plants derived from the colorless, sh_2, y class of kernels, 6 had a single Spm element (C, table 2), and 18 had no Spm.

Each of the 24 plants derived from the uniformly pigmented kernels was tested for presence or absence of Spm in the described manner and in none of them was Spm found to be present. All of the plants derived from the variegated kernels showed streaks of the A_1 type pigment in a non-pigmented background and the test crosses indicated the presence of Spm in each of them. In 16 of the 17 plants derived from the variegated Sh_2 Y class of kernels, one Spm element was present and in 15 of these plants, it was linked with Y (D, table 2). In one

plant, however, no evidence of linkage of the single Spm element with Y was noted (E, table 2). In the remaining plant in this group, 2 Spm elements were present, neither of which was linked with Y (F, table 2). More than one test cross ear was obtained from 11 of these 17 plants and the number and location of the Spm element was the same in the cells producing all ears except for one plant. In this plant, one Spm element was present in the cells producing the main ear and it was linked with Y (G-1, table 2). In the cells that produced the tiller ear, however, a single Spm element was present but it showed no linkage with Y (G-2, table 2

(4) In all 8 plants derived from the variegated Sh_2 y class of kernels, one Spm was present (H, table 2). One Spm element was also present in the plant derived from the variegated sh_2 Y kernel. This plant was used as a pollen parent in crosses with plants having different constitutions: (1) homozygous for a_1 and sh_2/and also for y and having no Spm, (2) homozygous for several different states of a_1^{m-1} but having no Spm, and (3) ~~to plants heterozygous for different states of a_1^{m-1} that were~~ a_1^{m-1} Sh_2/a_1 sh_2, y/y, no Spm. In this third group, plants with different states of a_1^{m-1} were represented. All tests indicate the presence in the pollen parent of a_1^{m-1} in one sh_2 carrying chromosome and of Spm in the Y carrying chromosome, and that this Spm element was capable of activating the various different states of a_1^{m-1}.

The tests described above were conducted with the progeny of a single plant in a culture. There were 19 variegated plants in this culture. Each was derived from a variegated kernel that appeared on an ear of a variegated plant that was a_1^{m-1} Sh_2/ a_1 Sh_2, Y/y, wx/wx (chromosome 9), pr/pr (chromosome 5) in constitution when pollen of a plant homozygous for a_1, sh_2, y, Pr, and Wx and having no Spm had been placed on the silks of this ear. All kernels appearing on t is ear were Sh_2 and the distribution of phenotypes among them were as follows: 28 uniformly dark pale Y, 7 uniformly dark pale y, 55 variegated (spots of deep pigmentation in a colorless background) Y, 86 variegated y, 75 colorless Y, and 103 colorless y. Among the kernels showing anthocyanin pigment, the ratio of uniformly dark pale colored kernels to variegated kernels indicated the presence of at least 2 and possibly 3 Spm elements in the pistillate parent plant and one of these appeared to be linked with Y. The silks of ears of 9 plants derived from the variegated Y class of kernels on t is ear and of 10 plants derived from the variegated y class received pollen from plants that were homozygous for a_1, sh_2, and y, and had no Spm. The ratio of kernel types appearing on the resulting ears produced by each of these 9 plants is entered in table 3. In this table, the 9 plants are placed in four groups, A to D, according to the assumed constition of Spm in each that the ratio of

kernel types suggested. The 6 plants in A of this table were assumed to have a single Spm element located in the Y bearing chromosome. Progeny from 4 of these 6 plants were grown and again tested for Spm. Those derived from plant 6629A-1, line 1 of A of table 3, were considered separate above. The total number of progeny plants in this group that were tested and their phenotype of the kernel from which they arose are entered in the last line of A of table 3. These tests verified the presence of Spm in the variegated kernels and plants and its absence in the uniformly pigmented kernels and plants. They also verified that assumed Spm constitution and location in the pistillate parent plant. However only those tests conducted with the 116 plants derived from the colorless sh_2 class of kernels will be summarized here. Among the 56 plants derived from the a_1 sh_2 Y class of kernels, 32 carried Spm and 24 had no Spm. In 30 of the 32 plants having Spm, linkage of it with Y was expressed, I, table 2. In two plants, the Spm element did not appear to be linked with Y, as mentioned earlier (B, table 3). Among the 60 plants derived from the a_1 sh_2 y class of kernels, 17 had a single Spm element (J, table 2) and 43 had no Spm.

In order to verify the absence of linkage of Spm with Y ~~given Spm constitution and location~~ in the 2 plants entered in B of table 2, tests of some of the progeny of both of them were conducted. An ear of one of these plants had been self-pollinated

and another ear of this plant had been used in the cross with an a_1^{m-1} Sh_2 y, no Spm tester plant. The progeny from both of these ears were again tested for Spm constitution and location. The silks of ears of 8 plants derived from a_1 sh_2 kernels in the Y class on the self-pollinated ear received pollen from the a_1^{m-1} Sh_2 y, no Spm tester plants. From the kernel types on the resulting ears it could be concluded that ~~two~~ 2 of these 8 plants were Y/Y in constitution and that one of them had no Spm whereas the other had 1 Spm (199 pale colored kernels : 169 variegated kernels on the test cross ear). The remaining 6 plants were Y/y. One Spm was present in 4 of them but it was not linked with Y (K, table 2). An Spm element appeared to be carried at allelic positions in a pair of homologous chromosomes in the remaining 2 plants (L, table 2). Seventeen plants derived from the variegated Sh_2 Y class of kernels on ~~1~~ 10 testcross ear of this same parent (column 6, K, table 2) plant were crossed by plants in the Spm tester stocks. In 16 of these 17 plants, one Spm was present and on none of the ears produced by 15 of them was their any evidence of linkage of Spm with Y (M, table 2). However, the ratio of kernel types appearing on the test cross ear of one of them suggested such linkage (N, table 2). The remaining plant had 2 Spm elements, neither of which was linked with Y (O, table 2).

Tests of Spm constitution in 9 plants derived from the variegated Sh_2 Y kernels on the ear produced by the test cross with the other plant entered in B of table 2, suggested that the Spm element in this plant had been carried in the Y bearing chromosome but at a new location that was farther removed from Y. Seven of the 9 progeny plants had a single Spm element and in all of the test cross ears they produced, loose linkage of Spm with Y was expressed (P, table 2). One plant of the 9 had 2 Spm elements (Q, table 2) and the remaining plant had 3 Spm elements (R, table 2).

Further examples of the progeny test method of determining Spm constitution and location will not be given here. It should be mentioned, however, that such tests were conducted with the indicated progeny of the plants entered in B, C, and D of table 3, and also with the progeny of the 10 other plants of this same culture. Also, many additional tests were conducted with the progeny of plants having other states of a_1^{m-1} or combinations of states, i.e., carrying different states of a_1^{m-1} in each chromosome 3. All these tests clearly supported the 4 statements given on page , and particularly statements (1) and (2). More direct support for statements (3) and (4) was obtained by other tests to be described shortly. Various different locations of Spm, either in different chromosomes of the complement or in different locations within the same chromosome, were

discovered in these tests and each determined position of it was subsequently verified by means of progeny tests. However, it is clear from the tests so far described that Spm does not remain at one location within the chromosome complement but disappears from a known location and appears at a new location and this will be considered in a separate section. Before this is ~~described~~ discussed, it is necessary to show that the Spm element, regardless of location, is capable of acting upon any one of the states of a_1^{m-1}.

Responses of different states of a_1^{m-1} to the same Spm element

Evidence for statement (3)+(4), page , appeared in all tests in which either one of the two states of a_1^{m-1}, present in the tester stocks, had been used in crosses with plants carrying Spm. Each state responded to Spm in its characteristic manner. In order to determine whether the Spm element present in a particular plant would be capable of activating other states of a_1^{m-1}, several additional types of test were performed. One of them utilized different ears produced by a single plant. In one such test, these plants were selected that were homozygous for a_1. Spm could be either present or absent in any one of them. Pollen from a plant of the tester stock carrying either state s7118 - or state s7118-1 - was placed on the silks of an ear of one such plant. The silks of a second ear of the same plant received pollen from a plant that was homozygous for a different state of a_1^{m-1} and in which no Spm was present. If the pistillate plant had no Spm, then all kernels on both ears were uniformly pigmented, and the intensity of this reflected the state of a_1^{m-1} introduced by the pollen parent (excluding state s7120 -, figure 1, which gives colorless kernels in the absence of Spm). If, however, the pistillate parent carried Spm, then both variegated and non-variegated kernels appeared on each ear, but the phenotypes of the two classes of kernels on a third ear reflected the state of

a_1^{m-1} introduced by the pollen parent. Again, if the Spm element showed linkage with a genetic factor among the kernels on one ear, the same linkage was usually expressed on the other ear, and an example of this is given in 5 of table 2. In this test, the main ear of an a_1/a_1, Y/y plant received pollen from one of the Spm tester stocks. The kernel types on the ear this cross produced, 5-1, table 2, indicated the presence in the pistillate parent of an Spm element carried in the Y chromosome. The silks of a tiller ear of this same plant received pollen from a plant that was homozygous for state [illegible], figure 1, and also for y. The pollen parent was uniformly pigmented indicating the absence of Spm in it. The kernel types on the ear this cross produced are entered in 5-2 of table 2. The ratio of variegated to non-variegated kernels in the Y and y classes was much the same on both ears. Thus, it could be concluded that the Spm element, carried in the Y chromosome of the pistillate plant, was capable of activating either state of a_1^{m-1}. Pollen from the same collection that was used in the latter cross was also placed on the silks of a plant homozygous for a_1 and for y, but in which Spm was known to be absent. All of the 294 kernels on the ear this cross produced were uniformly lightly pigmented and all were y. This test confirmed the absence of Spm in the pollen parent.

Another type of test that was employed to indicate the response of different states of a_1^{m-1} to the same Spm element, utilized the pollen of plants that were homozygous for a_1 and in which one Spm element was present. The types of test conducted with two such plants, number 6861-1 and 6861-7, are illustrated in table 4. Both of these plants were homozygous for a_1 and sh_2, ~~and a single Spm element was present in each as shown by the ratios appearing in the table.~~ The silks of an ear of each plant received pollen from the a_1^{m-1} tester stock that carries state 5718. The types of kernels that appeared on the resulting ear are entered in A of table 4. These ratios indicate the presence of one Spm in each of these two plants. Both plants were used as pollen parents in crosses with plants that were homozygous for state 5719A-1 but in which no Spm was present. The types of kernels on the ears resulting from these crosses are entered in B of table 4. Again, a 1 : 1 ratio of variegated (Spm) to non-variegated (no Spm) kernels appeared on these ears, indicating the presence of 1 Spm in each of the two pollen parents. These same two plants were also used as pollen parents in crosses with plants that were a_1^{m-1} Sh_2/ a_1 sh_2 in constitution and had no Spm. The state of a_1^{m-1} in these latter plants was that which gives no anthocyani pigmentation in the kernel and plant in the absence of Spm (state 5720, - figure 1) but produces many mutations to the lower alleles of A_1 in its presenc

Other ears of these same plants received pollen from plants that were homozygous for a_1 and sh_2 but had no Spm. The types of kernels appearing on the ears resulting from each of these two types of cross are entered in C of table 4. Again, it is evident that plants 6861-1 and -7 each have one Spm element that is capable of acting on this state of a_1^{m-1}. The results obtained from the described tests are those to be expected if the Spm element in plants 6861-1 and -7 is capable of acting on different states of a_1^{m-1}. The same type of test as that just described was conducted with a_1 sh_2/a_1 sh_2 plants having more than one Spm element and the ratio of kernel types on the test cross ears was that expected if each of the Spm elements present in the a_1 sh_2/a_1 sh_2 plant was capable of acting on each of the states of a_1^{m-1}.

Still other types of test were conducted to determine the capacity of a Spm element to act on different states of a_1^{m-1}. One of them utilized the pollen of a plant that was a_1^{m-1} sh_2/a_1 sh_2 in which a single Spm element was present at a known location in the chromosome complement. When such a plant was used as a pollen parent in crosses to plants that were a_1^{m-1} $Sh_2/$ a_1 sh_2 and having no Spm but among which different states of a_1^{m-1} were represented, the types of kernels on the resulting ears clearly indicated the capacity of the Spm element in the male parent to act not only upon the state

of the a_1^{m-1} locus delivered by the male parent, but also upon the state of the a_1^{m-1} locus delivered by the female parent. Again, when plants carrying Spm that were a_1^{m-1} Sh_2/ a_1 sh_2, among which different states of a_1^{m-1} were represented, were used as pistillate parents in crosses with a plant that was homozygous for one state of a_1^{m-1} and also for sh_2 but in which no Spm was present, activation of the a_1^{m-1} state delivered by the male parent by the Spm element delivered by the female parent was indicated in all tests of this type.

Detailed consideration of the various types of test mentioned above cannot be given here. However, all of them clearly established the similarity of the Spm element carried in the many different tested plants, and regardless of its number or its location in the chromosome complement of a given plant. They also established the ability of the Spm element to act upon any of the selected states of a_1^{m-1} and they indicated that control of type of gene action in the absence of Spm and control of the time and frequency of occurrence of mutation as well as its type in the presence of Spm is solely a function of the state of a_1^{m-1}.

Pg. 11 - "Table 1 records the range of differences that is expressed among presently isolated state of Arm-1

Table-2

Phenotypes of kernels appearing on ears of plants having constitutions entered in column 2 when pollen of plants having constitutions entered in column 3 was placed on silks of these ears.

	Constitution of pistillate parent.		no. of plants	Phenotype of Kernel: uniformly pigmented (no Spm)		spots of deep pigment on colorless background (Spm)		
				Y	y	Y	y	Total
A	$a_1 sh_2/a_1 sh_2$; Y Spm	y +	13	813	1467	1386	815	4481
B	" "	Y/y;	1 Spm	96	97	126	116	435
C	" "	y/y;	1 Spm	6	1260	—	1216	2476
D	$a_1^{m1} sh_2/a_1 sh_2$; Y Spm	/y +	15	665	1156	1080	638	3539
E	" "	; Y/y;	1 Spm	43	47	31	35	156
F	" "	"	2 Spm	45	58	119	115	369
G-1	" "	Y Spm/y +	main ear	40	74	71	41	226
G-2	" "	Y/y; 1 Spm.	Tiller	122	129	144	121	516
H	" "	y/y	1 Spm	8	864	—	852	1716
I	$a_1 sh_2/a sh_2$	Y Spm/y +		30	1366	2619	2472	1335	7792
J	" "	y/y	1 Spm	17	—	3465	—	3281	6746
K	" "	Y/y	1 Spm	4	477	500	455	462	1894
L	" "	"	Spm/Spm	2	3	4	259	281	547
M	" "	"	1 Spm	15	1536	1664	1544	1654	6398
N	$a_1^{m-1} sh_2/a sh_2$	Y Spm	y + (?)	1	68	92	82	61	303
O	"	" Y/y	2 Spm	1	82	75	203	201	561
P	"	" Y Spm	/y +	7	581	713	628	543	2466
Q	"	" Y/y	2 Spm	1	51	71	170	175	467
R	"	" Y/y	3 Spm	1	16	15	95	110	236
S-1	$a_1 sh_2/a_1 sh_2$	Y Spm	y + (main ear)	1	67	130	141	78	416
S-2	" "	"	" (Tiller ear)		60	140	122	91	413

Tables

$a_1 a_2 Sh_2 / a_1 a_2 sh_2$; Y/y ♀ × $a_1 a_2 sh_2 / a_1 a_2 sh_2$; y y; no Spm ♂

	Phenotype of Kernel												
	Dark colored aleurone				spots of deep color in colorless background				Colorless aleurone				
	Sh_2		sh_2		Sh_2		sh_2		Sh_2		sh_2		Total
A. Ysm/y+ plant no. in culture	Y	y	Y	y	Y	y	Y	y	Y	y	Y	y	
6629 A-1	69	112	0	0	117	71	1	1	1	0	186	187	745
" A-3	34	52	0	1.	43	37	1	0	0	0	85	83	336
" A-4	23	65	0	0	56	36	0	0	0	0	90	84	354
" A-6	34	67	0	0	78	37	1	0	0	0	86	113	416
" A-7	29	59	0	0	58	36	0	0	4	1	105	100	392
" A-9	80	99	1	1	99	72	0	0	0	1	158	205	716
Totals	269	454	1	2	451	289	3	1	5	2	710	772	2959
Derivation of progeny plants tested in Spm.	35	28		2	43 (1 poor seed)	23	3				56	60	250
B. Ysm Spm/ y —													
6629 A-8	19	56	0	0	66	53	0	0	0	0	101	87	382
C. Ysm/y+ plus 2 Spm													
6629 A-2	10	27	0	0	123	118	0	2	1	0	128	116	525
D. Y/y plus 1 Spm													
6629 A-5	45	52	0	0	122	122	1	0	0	0	188	154	684

Table 4

A. $a_1 sh_2/a_1 sh_2$; 1 spm ♀ × $a_1^{wi} Sh_2/a_1^{wi} Sh_2$ (stock 5718); no Spm ♂

Phenotype of Kernels.

Plant number of ♀	uniform light pale	Dots of deep pigmentation in colorless background.	Total
6861-1	229	174	403
6861-7	139	148	287

B. $a_1^{wi} Sh_2/a_1^{wi} Sh_2$ (stock 5719A-1) ♀ × 6861-1 and 6861-7: $a_1 sh_2/a_1 sh_2$; 1 Spm ♂
no Spm

Phenotype of Kernels.

Number of ♀♀ tested	of parent	uniform dark pale	Areas of deep pigmentation in colorless background	Total
4	6861-1	788	779	1567
5	6861-7	988	988	1976

C $a_1^{wi} Sh_2$ (stock 5720)/$a_1 sh_2$ no Spm ♀ × $a_1 sh_2/a_1 sh_2$ 1 Spm (plants 6861-1 or 6861-7) and $a_1 sh/a_1 sh_2$ no Spm ♂

Phenotype of Kernels

♀♀ Tested	of	Colorless		Areas of light pigmentation in colorless background		Totals
		Sh_2	sh_2	Sh_2	sh_2	
6 plants	6861-1	530	951	445	0	1926
5 of above 6 plants	$a_1 sh_2/a_1 sh_2$; no Spm	741	795	0	0	1536
10 plants	6861-7	886	1671	798	1	3356
8 of above 10 plants	$a_1 sh_2/a_1 sh_2$; no Spm	1154	1177	0	0	2334

Stability of mutants produced by a_1^{m-1}

Mutation producing events may occur at a_1^{m-1} not only in somatic cells of the plant and in the endosperm cells of the kernel, but also in ancestor cells of the gametes, that is, in the sporogenous or gametophytic cells. The frequency of their occurrence in these latter cells, and the phenotypic expression that will result from this, is related directly to the state of the a_1^{m-1} locus itself (see table 1). With most states, the majority of germinal mutations modify the locus in such a way that it is subsequently capable of acting much like the standard A_1 locus. However, all states give rise to some germinal mutations that express a much reduced capacity for pigment production. State -(5720), figure 1, produces almost exclusively this latter type of mutant. When plants carrying a_1^{m-1} and Spm are crossed by plants that are homozygous for a_1, some kernels on the resulting ear may exhibit a modified phenotype and this is often the consequence of a germinal mutation at a_1^{m-1}. ~~These~~ Such kernels are uniformly pigmented and the intensity of this usually differs markedly from that appearing in the kernels having an ~~u~~unmodified a_1^{m-1} locus and no Spm. ~~K~~Some kernels exhibiting ~~expected from germinal mutations~~ phenotypes than were removed from ~~some of the~~ ears, and the plants grown from them were examined for anthocyanin distribution and were tested for presence or absence of Spm. It was found that ~~the intensity~~

these kernels gave rise to plants that were uniformly pigmented. When, in turn, those plants were crossed by plants that were homozygous for a_1^{m-1} but had no Spm, it was learned that Spm was present in some of them and absent in others. However, in all tests, the phenotype produced by the mutated locus was the same in all kernels carrying it. In other words, the action of the locus was not altered by the presence of Spm. ~~remained unchanged and segregated quite normally on these ears.~~ These plants were also crossed by plants carrying Spm and from this test it was learned that also the mutant locus in those plants that had no Spm would remain unaltered in expression when Spm was introduced.

The most graphic illustration of stability of mutants in the presence of Spm is derived from crosses of plants carrying the state of a_1^{m-1} (spro) that produces no anthocyanin in the absence of Spm but gives rise to many early occurring mutations to low alleles of A_1 in its presence. When this state is present, the frequency of occurrence of germinal mutations is high, and they are revealed by the appearance of kernels exhibiting a uniform distribution of pigment over the aleurone layer. The intensity of this pigment among the different kernels having germinal mutants varies from very faint in some to rather dark pale in others. The same range in intensity of pigmentation is expressed in the plants grown from these kernels, and the degree of this corresponding with that ~~shown~~ shown by the kernel from which the plant arose. These plants, in turn,

(homozygous for a_1^{m-1}, and lacking Spm)

were crossed with ~~the~~ an Spm tester stocks in order to determine the presence or absence of Spm in them. ~~and~~ Spm was found to be present in some of them. The stability of the mutant in the presence of Spm was clearly revealed in this test cross. Some of the kernels on the ~~xxxx~~ ear resulting from this cross ~~carried~~ received the mutated locus and Spm ~~derived~~ from one parent and the a_1^{m-1} locus ~~derived~~ from the other parent. ~~In~~ These kernels exhibited spots of deep pigmentation in a uniformly pale colored background, figure 3. The deeply pigmented spots represent Spm induced mutations at the a_1^{m-1} locus contributed by the tester stock. The lightly pigmented background in which these appear reflects the action of the mutated locus contributed by the other parent, ~~which is~~ Obviously ~~stable in~~ its expression is unaffected by the presence of Spm.

Types of Spm elements

The phenotypic expression of a_1^{m-1} produced by the Spm element, considered in the previous sections, was remarkably constant and predictable, notwithstanding the many different locations of it that were determined and changes in these that were detected. However, Spm elements with modified types of ~~expression~~ action have appeared and the origin and expression of one type will be considered here. Occasionally, on the ear of an a_1^{m-1}, Spm carrying plant, a kernel ~~with~~ exhibiting an aberrant phenotypic ~~expression~~ and one type has appeared rather frequently will appear, Instead of showing a number of deeply pigmented spots in a colorless background, such kernels show only a tiny spot or several such spots in a colorless background. Plants ~~have been~~ were grown from several such kernels and they and their progeny were tested to determine the ~~cause of~~ reason for the altered phenotypic expression. These tests have shown that in ~~such~~ some of these kernels, an Spm-type element is present but its capacity to suppress gene action at a_1^{m-1} and to induce mutations at this locus is much weakened. It has therefore been symbolized as Spm-w, ~~in contrast to the standard Spm element involved in the tests previously described~~.

In this section, the standard Spm element will be designated Spm-s to distinguish it from the Spm-w element.

Spm-w elements have been located in several different chromosomes. The one which will be considered ~~here~~ arose ~~in~~ has appeared in a single kernel on an ear produced by a plant carrying Spm-s in chromosome 5. This plant was $a_1^{m-1} Sh_2$ (state 2719A-1, figure 1)/ a_1 sh_2,

Pr-/pr Spm-s, y/y, wx/wx in constitution, was crossed by a plant that was homozygous for a_1, sh_2, y, Pr, and Wx and had no Spm. On the resulting ear there were 87 uniformly pigmented Sh_2 kernels (no spm), 103 Sh_2 kernels that had a number of deeply pigmented spots in a colorless background (spm-s present), and 41 Sh_2 kernels that showed only several small dots of deep pigment in a colorless background. In addition, there were 186 sh_2 kernels that were totally colorless. Progeny was grown from 3 expected classes of kernels and tested for presence or absence of Spm. From such tests it was possible to learn of the presence of Spm-s in the pr carrying chromosome of the variegated parent plant. Both plants derived from the Sh_2 kernels that showed only several tiny spots of deep pigment in a colorless background was uniformly pigmented and in this respect, it resembled the plants that had no Spm. Two ears of this plant were used in test crosses. Pollen from a plant homozygous for a_1, sh_2, y, pr, and Wx and having no Spm was used on the silks of one ear and pollen of a plant that was Y/y but homozygous for a_1^{m-1} (state-, figure 1), Sh_2, pr, and wx and having no Spm was used on silks of the second ear. The kernel types on the resulting ears indicated that the constitution of the tested plant was a_1^{m-1} Sh_2/a_1 sh_2, Wx/wx, Pr/Pr, y/y. However, kernels exhibiting the same phenotype as that from which the plant arose segregated on each of these ears. Among the 328 kernels

appearing on the ear produced by the latter cross, 169 were uniformly pigmented (no ^{S}pm type), and 78 of these were Wx and 91 wx. There were 118 kernels that had the same phenotype as that from which the parent plant arose (57 Wx : 51 wx) and in addition, there were 51 totally colorless kernels (27Wx: 24 wx). In order to determine the factors responsible for the modified type of expression of a_1^{m-1}, plants were grown from 3 classes of kernels on this ear and those appearing, delivered by the male parent, were selected. At maturity, the phenotypes of the plants derived from all three classes of kernels were alike. Each was uniformly pigmented. The silks of an ear of each of these plants received pollen from a plant that was homozygous for a_1, sh_2, y, and had 1 ^{S}pm-s element, closely linked with y in one of the two chromosomes 6. Some of the pollen parents were Pr/pr and others were pr/pr. From these tests it was concluded that an Spm element with much weakened action was present in each of the plants derived from the kernels that were eaither totally colorless or showed only 1 or several small spots of deep color in a colorless background, and that this element was absent in the plants derived from the uniformly colored kernels. The reason for this conclusion is evident from the types of kernels that appeared on the test-cross ears that these plants produced, as shown in table (a). Those entered in A of this table are from ears of plants derived from the uniformly colored

kernels. Those entered in B ~~of this table~~ are d~~xxxxxxxxxxxxxxxxxxxxxxxx~~ from ears of plants derived from the totally colorless ~~k~~ernels or those in which only ~~a~~ 1 or several small spot deep color appeared. Only two classes of a_1^{m-1} carrying kernels appeared on the ears of plants entered in A ~~of the table.~~ Half of them were uniformly dark pale in color (no Spm) and half exhibited many spots of deep pigmentation in a colorless background (Spm-s present). On the other hand, the a_1^{m-1} carrying kernels on ears produced by plants entered in B of this table fell into four classes. Half of them (1323 kernels) exhibited the typical pattern of variegation produced when Spm-s is present. A quater of them (629 kernels) were uniformly dark pale in color (no Spm). The remaining quater (693 kernels) showed either a few dots of deep pigmentation in a colorless background (524 kernels) or they were totally colorless (169 kernels). In some of the crosses entered in ~~B~~ this table, the male parent was pr/pr and when these ~~plants~~ were used ~~as pollen parent~~ in the crosses entered in B, of Table a, the distribution of Pr to pr among the three classes of a_1^{m-1} Sh_2 carrying kernels indicated ~~xxxx~~ the presence in the pistillate parent of a factor, carried in the pr chromosome, that is responsible for the ~~phenotype~~ colorless kernels exhibiting very few or no A_1 dots. These ratios, ~~are~~ of Pr to pr in each of the a_1 + classes exhibiting pigment, are entered at the foot of B of table (a). They ~~also~~ indicated that ~~xxxxxxxxxx~~ the Spm-s element, introduced by the

pollen parent, was epistatic to this Spm-w element factor. One of the plants belonging to group B of table (a) had been crossed by a plant homozygous for a_1^{m-1} Sh_2, y, and pr and having no Spm. The types of kernels this cross produced also indicated the presence of the weakened Spm-type factor that was carried in the pr chromosome. There were 253 uniformly pigmented kernels of which 70 were Pr and 183 were pr; 146 kernels showed 1 or several small A_1 dots in a colorless background and 112 of these were Pr and 34 were pr. In addition, there were 69 totally colorless kernels.

Another series of progeny tests were conduced with plants derived from the several classes of kernels on the ear just described and also from kernels entered in B of table (a). These progeny tests confirmed the conclusions derived from the tests just described. Details regarding these tests will not be given here but in order to indicate the obviousness of the conclusions, data from test crosses of some of these plants are given in table (b).

It was learned from these studies that the Spm-w element behaved as a weakened Spm element both with regard to suppression of pigment formation at a_1^{m-1} and with regard to mutation producing capacity. When it is present, the plants having a_1^{m-1} develop pigment but the rate of this is very much slower than in the a_1^{m-1} plants that have no Spm element. Also, suppression of pigment formation in the kernel is not always complete for a very

coloration may appear at the base of some of the kernels that have Spm-w. In order to determine if the Spm-w element has a weakened capacity to induce mutations, it was incorporated into plants having various different states of a_1^{m-1} but no Spm-s. These tests indicated that the presence of Spm-w results in a marked reduction of the frequency of occurrence of mutation but does not alter the time of their occurence. This latter remains a function of the state of the a_1^{m-1} locus.

Although Spm-w elements residing in different chromosomes of the complement have been detected, no certain evidence of transposition of this element has yet been obtained. Tests of this are not extensive, however, and thus no conclusions regarding this may yet be drawn. The origins of Spm-w elements from modifications of Spm-s elements is to be suspected but this conclusion could only be considered as tentative since evidence in support of it is limited to two cases where Spm-w elements appeared in the same chromosome that had carried Spm-s, and the that value adequate examples. In each of these two cases, however, the it showed the same linkage relations with a genetic factor in this chromosome as Spm-s had shown.

(...) Table

6888

$a_1^{m1}Sh_2/a_1^{m1}Sh_2$ or $a_1^{m1}Sh_2/a_1 sh_2$ ♀ × $a_1 sh_2/a_1 sh_2$ 1 Spm of Y/y
Pr/pr Y/y Pr/pr or pr/pr

Plants
A. From pale kernels.

Plant	Constitution		Sh_2 uniform pale	many A_1 areas in colorless background	few A_1 dots in colorless background	Totally colorless	sh_2 colorless sh_2	$a_1 \to A_1$ sh_2	Totals
A-3	$a_1^{m1}Sh_2$	$a_1^{m1}Sh_2$	214	183	0	0	—	—	397
B-1	"	"	205	288	1	0	—	—	494
A-1	"	$a_1 sh_2$	121	136	1	0	288	0	546
A-4	"	"	108	102	0	0	189	0	399
A-5	"	"	113	121	0	0	200	0	434
B-2	"	"	134	147	0	0	257	0	538
B-3	"	"	120	91	0	1	208	0	420
Totals			1015	1068	2	1			

B. From colorless [illegible] plants A_1 dots or totally colorless

Plant	Constitution		pale	many A_1	few A_1 dots	Totally colorless	colorless sh_2	$a_1 \to A_1$ sh_2	Totals
C-3 I+II	$a_1^{m1}Sh_2$	$a_1^{m1}Sh_2$	131	261	82	69	—		543
D-1	"	"	137	274	117	20	—		548
C-4	"	"	91	249	95	39	—		474
C-2	$a_1^{m1}Sh_2$	$a_1 sh_2$	54	122	48	4	217	1	441
C-5 I+II	"	"	91	145	69	13	313	2	633
D-3	"	"	68	139	72	4	264	0	547
D-6	"	"	57	133	41	20	244	0	495
Totals			629	1323	524	169			
Totals from ♂ [illegible]			447 (176 Pr : 271 pr)	886	383 (260 Pr : 123 pr)				

453 Pr : 433 pr

[illegible] ♀ × $a_1^{m1}Sh_2$ pr no Spm ♂

Plant	pale	many A_1	few A_1 dots	Totally colorless
D-2	253 (70 Pr : 183 pr)	0	146 (112 Pr : 34 pr)	69

4 plants test cross
8 " " from 1 kernel

Selections for test mig :

6888C-3^{I} a,mi sh$_2$ / a,mi sh$_2$ Pr Spm-w / pr+ x y/y x a,sh$_2$ pr
[Culture 7262] y spm/y+

" C-3^{II} " " " " " x a,sh$_2$ Pr/pr
[Culture 7263] y spm/y+.

6888D-2 a,mi sh$_2$ / a,mi sh$_2$ Pr Spm-w / pr+ y/y x a,mi sh$_2$ pyest
no Spm; no Spm-w
[Culture 7264]

Origin -

6629B-⑤^{I} x Bellinger 1040 ① [1953]

a,mi sh$_2$ / a,sh$_2$ a$_2$ sh$_2$ y Pr/Pr

Pr+/pr Spm-0 y/y

Colorless, few Acids Pr y = 6683B ② [1954]
=

6683B ② x 6641A-5

a,mi sh$_2$ / a,sh$_2$ a,mi sh$_2$ / a,mi sh$_2$ ppr mix

Pr+/Pr Spm-w y/y no Spm.

y/y \/

Save 6888 cultures [1955]

Table (2)

7264

I $a_1 m_1 Sh_2 / a_1 m_1 Sh_2$; Pr Spm-w / pr + × $a_1 m_1 Sh_2 / a_1 m_1 Sh_2$; pr/pr no Spm

II 7262 " " × $a_1 Rh_2 / a_1 Rh_2$; pr/pr, y Spm-o / y +

III 7263 " " " × " " ; Pr/pr " "

A. Plants from dark pale kernels. I = 5, III = 10 } Total = 15. No Spm-o ; no Spm-w

B. Plants from colorless kernels with 1 or several A, dets. Pr y + $a_1 m_1 Sh_2$ y pr no Spm-?

No. of plants tested	Constitution of tested Plant	Phenotype of Kernel: uniform dark pale		colorless with 1 or several A, dets.		colorless	Totals
		Pr	pr	Pr	pr		
I, 9	Pr Spm-w / pr +	431	1207	702	232	602	3,174
II = 5	Pr Spm-w / pr +	206	769	437	116	390	1918
III = 5	Pr Spm-w / pr +	298	782	403	138	457	2078
Totals " "		935 (25.3%)	2758	1542	486 (23.9%)	1449	7170
III = 2	Pr + / pr - Spm-w		203	124	229	323	1354
IV = 2	Pr / Pr Spm-w	284	—	143	—	142	569

Kinds - 7264E ⑥

7263C-5, C-7

C Plants from kernels with many deep colored spots in colorless background (Spm-o), Y, Pr.

	Spm-o: many spots of deep color in colorless background				Spm-w; no Spm-o: 1 or several small dots of color in colorless background				Spm-w: Colorless		no Spm-S, no " w: Uniformly dark pale				Total
	Y		y		Y		y				Y		y		
	Pr	pr	Pr	pr	Pr	pr	Pr	pr	Y	y	Pr	pr	Pr	pr	
II 3: Y+/y Spm-o; III 1 Pr Spm-w/pr+	16	19	360	342	212	69	11	9	56	5	98	200	9	12	1418
II 1. Y+/y Spm-o; III 4 Pr/pr no Spm-w	31	33	300	287	0	0	0	0	0	0	287	233	47	51	1268
III 1 Y/y; 1 Spm-o (Pr Spm-w/pr +)	30	45	48	30	20	5	11	5	19	18	22 *	20	15 *	22	310
II 1. Y+/y Spm-o Pr/Pr no Spm-w	8	—	60	—	0	—	0	—	0	0	67	—	16	—	151
II 1 Y/y 3 Spm-o (Pr Spm-w/pr+)	98	54	97	96	3	4	0	0	6	1	13	25	1	1	399
III 1 Y+/y Spm-o + 1 Spm-o Pr/Pr no Spm-w	81	—	194	—	0	—	0	—	0	0	135	—	28	—	438

D. Plants from kernels with many deep colored spots in colorless background; y; Pr × 9.m1 sh2 y pr no Spm-o; no Spm-w ♂

			Pr	pr			Pr	pr					Pr	pr	
III 2 1 Spm-o Pr Spm-w/pr+			97	102			45	18		24			34	72	392
II 1 2 Spm-o " "			183	217			30	11		20			20	51	532
III 2 1 Spm-o Pr/pr no Spm-w			121	126			0	0		0			130	134	511
III 1 2 Spm-o " " "			162	135			0	0		0			50	57	404
I 1 1 Spm Pr/Pr " "			204	—			0	—		0			206	—	410

* different Pr + coloring in pale class.

[illegible]

Modifier element in the Spm system

In the course of a test undertaken to examine Spm number and location in the progeny of a plant carrying two Spm elements, both located in one chromosome 5, a kernel appeared on ~~a~~ one ear of a ~~single~~ plant in which the frequency of mutation at a_1^{m-1} was greatly augmented. Subsequent tests of the plant derived from this kernel and its progeny indicated the presence of an element, belonging to the Spm system, that increases the frequency of occurrence of mutation at a_1^{m-1} and with each of five tested states of At, but does not alter the time during ~~the~~ development when its presence may be detected only when Spm also is present. Several these will occur. / Like Spm, it may undergo transposition. In all essential respects, it acts like a complementary controlling element within the Spm system.

The kernel carrying the modifier element appeared on ~~the~~ one of two ears of a plant that was a_1^{m-1} Sh_2/a_1 sh_2, Pr/Pr, Wx/Wx ~~in constitution~~ when two of its ears each had been used in a cross with a plant that was homozygous for a_1, sh_2 pr, and Wx and had no Spm. / On these ears, there were 167 uniformly dark pale colored kernels in the Sh_2 class (no Spm), 186 Sh_2 kernels with spots of deep color (Spm present) in a colorless background with a pattern of mutant spots similar to that for it caused the state of a_1^{m-1}. shown in ~~7~~ CHGA-1 of figure 1. There were also 384 colorless, sh_2 kernels. In addition, on one of these ~~two~~ ears, a single Sh_2 kernel appeared that

exhibited a very marked increase in the number of pigmented spots. ~~This kernel was removed and the~~ plant grown from ~~it~~ her ear also exhibited a very high rate of mutation at a_1^{m-1} in its somatic cells. In order to examine the nature of the modification that was responsible for this marked increase in frequency of mutation, one ear of this plant,(number 6889), was self-pollinated. Another ear received pollen from a plant that was homozygous for a_1^{m-1} (state), Sh_2, pr, and wx. The reciprocal cross was also made. In addition, pollen from plant 6889 was placed on the silks of ~~ears of~~ two plants that were homozygous for a_1, sh_2, pr, and had not Spm. The kernel types and the ratios of them that appeared on the ears produced by these crosses indicated the presence of one Spm element in plant 6889 but it was not linked with Pr. Among the variegated kernels, there were two classes. In one, the kernels exhibited the expected number of mutant spots. In the other, one the other hand, the number of these spots was greatly increased. The ratios of these two types of variegated kernels suggested the presence in plant 6889 of an independently located modifer element that serves to increase the frequency of mutation at a_1^{m-1} and does so either with state or state . In order to verify this, kernels were selected from the various classes on these ears, and the plants grown from them were again tested for presence or absence of this modifier in accordanc

with the phenotype of the kernel from which the plant arose. These tests verified the presence of the modifier in those plants that were derived from the kernels showing an increased frequency of mutation and its absence in those plants that were derived from the kernels that exhibited the usual frequendy of mutation associated with the state of a_1^{m-1} present in the plant. They also showed that Spm was required for the modifier to be expressed. It was also possible to learn from these tests that the modifier undergoes transposition in somatic cells. This results in removal of it from some cells, and increase in its number in others. In one case, this resulted in its insertion into chromosome 9, and linkage of the modifier with Wx could be determined in plants having the modifier in this location. Removal from this location and insertion elesewhere coul also be followed. Detailed evidence for the statements given above cannot be included in this report. However, in order to indicate some of the methods used in obtaining this evidence, several of the tests will be outlined.

In the cross of plant 6889 to a plant that was homozygous for a_1, sh_2 and had no Spm, 12 plants derived from the variegated kernels showing the usual number of mutant spots (Spm, no modifier) were crossed by plants homozygous for a1, sh2 and having no Spm, and also by plants homozygous for

a_1^{m-1}, (state), Sh_2 and having no Spm. The types of kernels on the ears these crosses produced are entered in A and B of table . Eight plants derived from the variegated kernels with an increased number of mutant spots (Spm plus modifier) were also used in crosses of the same type. The types of kernels appearing on the ears resulting from the cross with the plants homozygous for a_1 and sh_2 are entered in C of table .

Because the kernels on the ears produced by the cross with the a_1^{m-1} Sh_2 no Spm tester plants have different states of a_1^{m-1} in them that would require additional categories of kernel types in the table, they have been excluded from it.

7267 Cultures

A $a_1 m sh_2 / a_1 sh_2$; Spm; no modifier ♀ × $a_1 sh_2 / a_1 sh_2$; no Spm; no modifier ♂
B. " " " ; modifier ♀ × $a_1 m sh_2 / a_1 m sh_2$ " " " ♂
C. " " " ; modifier ♀ × $a_1 sh_2 / a_1 sh_2$; no Spm, no modifier ♂

	number of plants tested. 7267	Number of ears	germinal mutants	mottled; dark pale		Areas of deep color in areas of pale background. (see figure)		Very many areas of deep colored in colorless background		Colorless		
				no Spm		Spm; no modifier		Spm, modifier				
			Sh_2	Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	Totals
A from pale kernels	1) C-7 D-3, 8	11	5	557	3	592	1	0	0	12	1141	2311
	2) Tiller C-7 =	1	0	28	0	169	1	0	0	0	206	404
	3) tiller D-5 = 7267 C-7; D-3,	1	0	95	0	0	0	0	0	0	114	209
B.	1) 11 1Spm	11	4	2013	–	1995	–	0	–	0	–	4012
	1) D-4 1 2Spm	1	2	66	–	253	–	0	–	0	–	321
C	1) (1Spm; 1 modifier A-3 B-1 B-5 - 3	6	4	372	0	165	0	187	1	3	729	1461
	2) 1Spm, 2 modifiers A-3 E-2 B-? 2	2	2	86	0	30	0	94	0	1	200	413

present in some of its gametes. Following introduction of the a_1^{m-1} locus from the male parent, the presence of Spm in those kernels that received it from the female parent should be revealed by the appearance in them of small deeply pigmented spots in a colorless background due to activation of the a_1^{m-1} locus by the Spm element. In those kernels that did not receive Spm, the aleurone layer should be uniformly pigmented. Among the 30 plants derived from the colorless, sh_2, Y class of kernels, it could be determined on this basis that 15 had a single Spm element and 15 had no Spm. In 13 of the 15 plants that had Spm, linkage of it with Y was evident (A, table 2) but in the 2 remaining plants, no linkage of Spm with Y was noted (B, table 2). (The reason for the absence of linkage of Spm with Y in these 2 plants will be considered in the next section. It need only be mentioned here that this is not unexpected.) Among the 24 plants derived from the colorless, sh_2, y class of kernels, 6 had a single Spm element (C, table 2), and 18 had no Spm.

In the above described test, the state of a_1^{m-1} present in the tester stock (pollen parent) was either that shown in - or - of figure 1. All of the kernels having Spm exhibited the pattern of variegation characteristic of the introduced state,-- small spots of deep pigmentation in a colorless background, and these spots were rather uniformly distributed

over the aleurone layer. Also, all those that had no Spm were uniformly pigmented either darkly pigmented if state - had been introduced by the male parent, or lightly pigmented if state - had been so introduced. More than one fertile ear was produced by some of these plants. This made it possible to place on the silks of some of these additional ears pollen of plants in which Spm was considered to be absent but in which other states of a_1^{m-1} were present. This was done with plants having the states shown in -, -,-, and - of figure 1. It was found that if the element present in the pistillate parent had activated the state of a_1^{m-1} present in the tester stock, it would also activate each of these other states of a_1^{m-1}. However, the pattern of variegation that appeared in the kernels that received Spm was not the same as that given by the tester stocks. Instead, it was that which characterized the particular state of a_1^{m-1} that had been introduced by the pollen parent. That the activating element, nevertheless, was the same for each state could be shown in some of these tests by means of its linkage with Y and an illustration of this is given in D of table 2. ~~A more direct~~ other tests which shows the activation of the different states of a_1^{m-1} by the same Spm element was conducted and the result of one of these ~~tests~~ is illustrated in figure 2. In this test, a variegated plant having the state of a_1^{m-1} shown in - of figure 1 was

was crossed by the Spm tester stock having the state shown in - of figure 1. Without exception, all kernels that exhibited the pattern of variegation characteristic of the state of a_1^{m-1} present in the female parent likewise exhibited the pattern of variegation characteristic of the state of a_1^{m-1} that was present in the male parent. The same element activated both states of a_1^{m-1}. Another type of test involved use of ~~pollen from a~~ plants that was homozygous for the standard recessive, a_1 in which ~~the~~ Spm ~~constitution~~ could ~~not be determined by phenotype of the plant~~. Pollen from ~~such a~~ plant was ~~placed upon~~ the silks of ~~a number~~ of non-variegated plants among which ~~a number of~~ different states of a_1^{m-1} ~~were represented~~. ~~From~~ each ~~such~~ test the ~~proportion~~ of variegated to non-variegated kernels was the same, regardless of the state of a_1^{m-1} that was present in the pistillate parent. ~~The proportion of these two classes of kernels indicated~~ the number of Spm elements in the pollen parent. Again, if linkage of Spm to a given marker was expressed among the kernels on one ear, it was also expressed in all ears where this could be detected.

Phenotypes of kernels appearing on ears of plants bearing constitution shown in column 1 when pollen of plants bearing genes for a, ai, sh2, + wx was placed on silks of these ears.

Table 2

Phenotypes of Kernels

Constitution of pistillate parent	plants each constitution	uniformly pigmented (no Spm)		spots of deep pigmentation in colorless background (spm present)			Total no. of kernels
		Y	y	Y	y		
A $a_1 sh_2/a_1 sh_2$ Y Spm/y +	13 plants	813	1467	1386	815		4481
B " " Y/y; 1 Spm	2 " (6665 G-16 " G-21)	96	97	126	116		435
C. " " y/y; 1 Spm	6 "	—	1260	—	1216		2476
D. $a_1 sh_2/a_1 sh_2$ Y Spm/y +	15 "	665	1156	1080	638		3539
E " " Y/y; 1 Spm	1 "	43	47	31	35		156
F " " Y/y; 2 Spm	1 "	45	58	119	115		369
G-① " " Y Spm/y + main ear		40	74	71	41		226
② " " Y/y; 1 Spm, tiller ear		122	129	144	121		516
H. " " y/y; 1 Spm	8	—	864	—	852		1716
I $a_1 sh_2/a_1 sh_2$ Y Spm/y +	30	1366	2619	2472	1335		7792
J. " " y/y 1 Spm	17	—	3465	—	3281		6746
K 6863 (6665 G-21) " " Y/y 1 Spm	4	477	500	455	462		1894
L. " " " Spm/Spm	2	3	4	259	281		547
M. 6867 (6665 G-21) " " Y/y 1 Spm	15	1536	1664	1544	1654		6398
N $a_1 sh_2/a_1 sh_2$ Y Spm/y + (?)	1	68	92	82	61		303
O. " " Y/y 2 Spm	1	82	75	203	201		561
P. 6866 (6665 G-16) $a_1 sh_2/a_1 sh_2$ Y Spm/y + (?)	7	581	713	628	543		2466
Q " " Y/y 2 Spm	1	51	71	170	175		467
R. " " Y/y 3 Spm	1	16	15	95	110		236
S 6665 G-1 main ear $a_1 sh_2/a_1 sh_2$ Y Spm/y + 6638 A-3 main ear 6701-2		67	130	141	78		416
S-2 " 6665 G-1 tiller ear " "		60	140	122	91		413
T "		—		—			

* a few kernel mutations not scored - too few.

Transposition of spm -

1. Parent plant - 1 spm; progeny possess with no spm of.
2. " " - 1 spm - linked - " " cross with no spm - Recombinants & non-recombinants
3. Reppeares in tillers of same plant - Linked
4. " " sectors in same starch-tests. Same ear

Ratios =

Plants from $a_1 \rightarrow A_1$ dues no spm : plants from pale dues = spm

Late transposition of spm .

	pale		var2		
	¼	µg	¼	µg	
6665G-1 + 6638A-3	67	130	141	78	✓ ✓
" G① + 6701-2	60	140	122	91	
" G③ + 6638A-3	200				
6630C-8 + 6701-2	—	294	—	0	✓
6666H-6 + 6638A-3	—	175	—	182	
" " + 6704C-4	—	72		86	

A. ♀	♂	pale sh_2	var. sh_2	Totals
$a_1 sh_2 / a_1 sh_2$ 1 Spm	$a_1^{m1} sh_2 / a_1^{m1} sh_2$ state 5718 no Spm			
6861-1		229	174	
6861-7		139	148	

B. ♀	♂			
$a_1^{m1} sh_2 / a_1^{m1} sh_2$ no Spm ×				
state 5719A-1 (4 plants)	5861-1	788	779	
" " (5 ")	5861-7	988	988	

C.		colorless sh_2	var. sh_2	var. Sh_2	colorless Sh_2	totals
1) $a_1^{m1} sh_2 / a_1 sh_2$ no Spm ×	$a_1 sh_2 / a_1 sh_2$ 1 Spm					
state 5720 (6 plants)	5861-1	530	445	0	951	
2) 5 of 6 above plants ×	$a_1 sh_2 / a_1 sh_2$ no Spm	741	0	0	795	
3) " " ×	$a_1 sh_2 / a_1 sh_2$ 1 Spm					
(10 plants)	5861-7	886	798	1	1671	3356
" (8 of above 10 plants)	$a_1 sh_2 / a_1 sh_2$ no Spm	1157	0	0	1177	2334

		pale	$a_1 \to A_1$
6861-1 x 6857-5 (stat[5718] –)		229	174
6859-14 x 6861-1 (stat –) 5719A-1	4 plants	174	180
		245	247
		195	188
		174	164
		788	779

Totals

6899A (stat –) [5720] x 6861-1		colorless Sh_2	$a_1 \to A_1 Sh_2$	$a_1 \to A_1 sh_2$	colorless sh_2
a_1 m Sh_2 / a_1 sh_2	A-3	86	74		152
	A-8	116	100		173
	A-9	86	68		154
	B-1	75	77		137
	B-4	60	62		152
	B-7	107	64		183
		530	445		951

975

6899A ⑧t x a_1 sh_2 no spm.	115	143
" ⑨t x " "	119	103
" B ①t " "	154	160
" B-④t x " "	234	256
" B-7 x " "	119	133
	741	795

		Pale Sh$_2$	non. Sh$_2$
6861-7 ♀ x 6857-5 (state -)		139	148
state - x 6861-7 a.sh$_2$/a.sh$_2$		157	119
		206	288
		267	258
		247	217
		111	106
	Totals	988	988

State 5720 a.wi Sh$_2$/a.sh$_2$ x 6861-7

		colorless Sh$_2$	non Sh$_2$	non sh$_2$	colorless sh$_2$	Total
6899 A - 2		128	114	1	211	454
" A - 4		107	80	0	196	383
" A - 6		100	93	0	199	392
" A - 7		120	117	0	228	465
" B - 2		97	83	0	169	349
" B - 5		100	110	0	196	406
" B - 6		77	71	0	144	292
" B - 8		24	21	0	60	105
" B - 9		73	60	0	150	283
" B - 10		60	49	0	118	227
	Totals	886	798	1	1671	3356

6899 x a.b2 mosaic

	cheiles Sh2	cheiles nb2	Totals
A2	55	69	124
A4	154	155	309
A6	143	152	295
A7	93	107	200
B-2	255	229	484
B-5	126	129	255
B-6	210	202	412
B-10	121	134	255
Totals	1157	1177	2334

A

Constitution of ♀	Constitution of ♂	Phenotypes of hemes: uniform pale colored	A. actinum colorless background	Totals
$a_1 sh_2/a_1 sh_2$ 1 spm	$a_1 mi\ Sh_2/a_1 mi\ sh_2$ no spm state 5718.			
Plant 6861-1		229	174	403
Plant 6861-7		139	148	287

B. $a_1 mi\ Sh_2/a_1 mi\ sh_2$ (state 5719A-1) no spm ♀ × $a_1 sh_2/a_1 sh_2$ 1 spm ♂; plants 6861-1 and 6861-7

			Totals
4 ♀ plants × 6861-1	788	779	1567
5 ♀ " × 6861-7	988	988	1976

C. $a_1 mi\ Sh_2$ (state 5720)/$a_1 sh_2$ no spm ♀ × $a_1 sh_2/a_1 sh_2$, no spm ♂ and $a_1 sh_2/a_1 sh_2$ 1 spm ♂

	Colorless Sh_2	area of light pigmented interlens background Sh_2	area of light pigmented in colorless background sh_2	colorless sh_2	Total
6 ♀ plants × $a_1 sh_2/a_1 sh_2$ 1 spm - Plant 6861-1	530	445	0	951	1926
5 ♀ above 6 plants × " " no spm	741	0	0	795	1536
10 ♀ plants × " " 1 spm ♂ Plant 6861-7	886	798	1	1671	3356
8 ♀ above 10 plants × " " no spm ♂	1157	0	0	1177	2334